

What Is Claimed Is:

1. A composition for quantifying or detecting one or more target nucleic acid molecules in a sample comprising one or more detectably labeled oligonucleotides and one or more target nucleic acid molecules to be detected or quantified, wherein said oligonucleotides comprise one or more detectable labels located internally and/or at or near the 3' and/or 5' termini of said oligonucleotides and wherein said label undergoes a detectable change in an observable property upon becoming part of a double stranded molecule.

2. The composition of claim 1, wherein said detectable change is an increase or enhancement in the level of activity of the detectable label compared to the level of activity of the detectable label in the absence of said target nucleic acid molecules.

3. The composition of claim 2, wherein said detectable labels are selected from the group consisting of fluorescent labels, chemiluminescent labels and bioluminescent labels.

4. The composition of claim 3, wherein the fluorescent label is selected from the group consisting of FAM, TAMRA, JOE, Rhodamine, BODIPY, R6G, ROX, and EDANS.

5. The composition of claim 1, wherein said one or more detectable labels are the same or different.

6. The composition of claim 1, wherein one or more of said oligonucleotides comprise one or more hairpin structures.

7. The composition of claim 1, wherein one or more of said oligonucleotides is hybridized to one or more of said nucleic acid molecules.

5 8. The composition of claim 1, further comprising at least one component selected from the group consisting of one or more nucleotides, one or more DNA polymerases and one or more reverse transcriptases.

9. The composition of claim 1, wherein said nucleic acid molecules are RNA and/or DNA molecules.

10 10. A method for the quantification or detection of one or more target nucleic acid molecules in a sample comprising hybridizing one or more detectably labeled oligonucleotides of claim 1 with one or more molecules to be detected or quantified, and detecting the presence or absence and/or
15 quantifying the amount of said target nucleic acid molecules.

11. A method for the quantitation or detection of one or more nucleic acid molecules in a sample during nucleic acid synthesis comprising:

mixing one or more nucleic acid templates with one or more
20 oligonucleotides of claim 1;

incubating said mixture under conditions sufficient to synthesize one or more nucleic acid molecules complementary to all or a portion of said templates, said synthesized nucleic acid molecule comprising said oligonucleotides; and

25 detecting the presence or absence or quantifying the amount of said synthesized nucleic acid molecules by measuring said detectable label.

12. A method for quantitation or detection of one or more nucleic acid molecules in a sample during nucleic acid amplification comprising:

mixing one or more nucleic acid templates with one or more oligonucleotides of claim 1 under conditions sufficient to amplify one or more nucleic acid molecules complementary to all or a portion of said templates, said amplified nucleic acid molecule comprising said oligonucleotides; and

detecting the presence or absence or quantifying the amount of said nucleic acid molecules by measuring the detectable labels of said oligonucleotides.

13. The method of claim 12, wherein said label is selected from the group consisting of fluorescent labels, chemiluminescent labels and bioluminescent labels.

14. The method of claims 11 or 12, wherein said detection step comprises detecting or measuring the level of activity of the detectable label during said synthesis or amplification compared to the level of activity of the detectable label in the absence of said synthesis or amplification.

15. The method of claim 12, wherein said amplification is accomplished by at least one method selected from the group consisting of PCR, 5-RACE, RT PCR, Allele-specific PCR, Anchor PCR, "one-sided PCR," LCR, NASBA, and SDA.

16. The method of claim 13, wherein said oligonucleotides comprise one or more fluorescent labels.

17. The method of anyone of claims 10, 11 or 12, wherein said one or more oligonucleotides comprise one or more hairpin structures.

18. A method for amplifying a double stranded nucleic acid molecule, comprising:

providing a first and second primer, wherein said first primer is complementary to a sequence within or at or near the 3'-termini of the first strand of said nucleic molecule and said second primer is complementary to a sequence within or at or near the 3'-termini of the second strand of said nucleic acid molecule;

hybridizing said first primer to said first strand and said second primer to said second strand in the presence of one or more of the polymerases, under conditions such that a third nucleic acid molecule complementary to all or a portion of said first strand and a fourth nucleic acid molecule complementary to all or a portion said second strand are synthesized;

denaturing said first and third strand, and said second and fourth strands; and

repeating the above steps one or more times, wherein one or more of the primers comprise a detectable label internally and/or at or near its 3' and/or 5' termini and/or comprises one or more hairpin structures.

19. The method of ~~claim~~ 18, wherein at least one of said primers comprises at least one hairpin structure.

20. A method for the quantification or detection of nucleic acids molecules comprising:

mixing one or more labeled oligonucleotides with one or more nucleic acid molecules to be detected or quantitated; and

detecting or measuring an increase in fluorescence associated with said oligonucleotide hybridizing to said nucleic acid molecules.

21. The method of claim 20, wherein the fluorescent label is FAM.

22. The method of claim 20, wherein the fluorescent label is TAMRA.

23. A composition comprising one or more nucleic acid molecules and at least one oligonucleotide, wherein at least a portion of said oligonucleotide is capable of hybridizing with at least a portion of said nucleic acid molecule and wherein said oligonucleotide comprises a specificity enhancing group.

24. The composition according to claim 23, wherein the group is a fluorescent moiety.

25. The composition according to claim 23, wherein the moiety is attached to a nucleotide at or near the 3'-most terminal nucleotide.

26. The composition according to claim 23, wherein the moiety is attached to one of the ten 3'-most terminal nucleotides.

27. The composition according to claim 23, wherein the moiety is detectable.

28. The composition according to claim 23, wherein at least a portion of said oligonucleotide is hybridized to at least a portion of said nucleic acid molecule.

29. The composition according to claim 23, wherein the oligonucleotide is capable of forming a hairpin.

30. The composition according to claim 23, wherein the oligonucleotide is in the form of a hairpin.

31. A method of making a composition, comprising the steps of:
providing at least one oligonucleotide; and

contacting said oligonucleotide with at least one nucleic acid molecule,
wherein at least a portion of said oligonucleotide is capable of hybridizing
5 with at least a portion of said nucleic acid molecule and wherein said
oligonucleotide comprises a specificity enhancing group.

32. The method according to claim 31, wherein oligonucleotide is
in the form of a hairpin.

33. A method of determining the presence of one or more
particular nucleotides at a specific position or positions in a target nucleic acid
molecule, comprising:

contacting at least one target nucleic acid molecule having one or more
15 nucleotides of interest at a specific position or positions on a target nucleic
acid molecule with at least one oligonucleotide, wherein at least a portion of
the oligonucleotide is capable of forming base pairs or hybridizing with at
least a portion of the target nucleic acid molecule and wherein the
oligonucleotide comprises at least one specificity enhancing group and/or one
20 or more hairpin structures; and

incubating the oligonucleotide and the target nucleic acid molecule
under conditions sufficient to cause extension of the oligonucleotide when the
3'-most nucleotide or nucleotides of the oligonucleotide base pair with the
nucleotide or nucleotides at the specific position or positions of the target
25 nucleic acid molecule, wherein the production of an extension product
indicates the presence of the particular nucleotide at the specific position.

34. The method according to claim ~~33~~, wherein the group is
attached to a nucleotide near the 3'-terminal nucleotide.

35. A method of determining the absence of one or more particular nucleotides at a specific position or positions in a target nucleic acid molecule, comprising:

5 contacting at least one target nucleic acid molecule having one or more nucleotides of interest at a specific position or positions on the target nucleic acid molecule with at least one

10 oligonucleotide, wherein at least one portion of the oligonucleotide is capable of forming base pairs or hybridizing with at least a portion of the target nucleic acid molecule and wherein the oligonucleotide comprises at least one specificity enhancing group and/or one or more hairpin structures; and

15 incubating the oligonucleotide and target nucleic acid molecule under conditions sufficient to inhibit or prevent extension of the oligonucleotide when the 3'-most nucleotide or nucleotides of the oligonucleotide does not substantially base pair with the nucleotide or nucleotides of the specific position or positions of the target nucleic acid molecule, wherein the lack of or reduced production of an extension product indicates the absence of the particular nucleotide at the specific position.

20 36. A method of determining the presence or absence of one or more particular nucleotides at a specific position or positions in a target nucleic acid molecule, comprising:

25 contacting at least first oligonucleotide with at least one target nucleic acid molecule under conditions sufficient to cause extension of the first oligonucleotide when the 3'-most nucleotide or nucleotides of the oligonucleotide base pairs with the nucleotide or nucleotides at the specific position or positions of the target nucleic acid molecule, wherein said first oligonucleotide comprises at least one specificity enhancing group and/or at least one hairpin structure;

contacting at least a second oligonucleotide with at least one target nucleic acid molecule under conditions sufficient to inhibit or prevent extension of the oligonucleotide when the 3'-most nucleotide or nucleotides of the oligonucleotide do not substantially base pair with the nucleotide or nucleotides at the specific position or positions of the target nucleic acid molecule, wherein said second oligonucleotide comprises at least one specificity enhancing group and/or at least one hairpin structure; and

comparing the level of extension or the amount of extension product accomplished with the first oligonucleotide compared to the second oligonucleotide.

37. The method of claim 33, wherein said conditions are sufficient to cause amplification of all or a portion of the target nucleic acid molecule.

38. The method of claim 35, wherein said conditions are sufficient to inhibit or prevent amplification of all or a portion of said target nucleic acid molecule.

39. The method of claim 33, wherein said conditions are accomplished in the presence of Tsp DNA polymerase.

40. The method of claim 35, wherein said conditions are accomplished in the presence of Tsp DNA polymerase.

41. The composition of claim 1, wherein said composition further comprises a quenching molecule.

42. The composition of claim 41, wherein said quenching molecule is selected from the group consisting of a single stranded binding protein and an oligonucleotide comprising at least one quenching moiety.

43. The composition of claim ~~42~~, wherein said oligonucleotide comprising at least one quenching moiety is capable of hybridizing to or base pairing with said detectably labeled oligonucleotides.

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44. The composition of claim 1, wherein said detectably labeled oligonucleotide further comprises one or more quenching moieties.

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45. The composition of claim 1, wherein said detectably labeled oligonucleotide comprises one or more hairpin structures and further comprises one or more quenching moieties.

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46. The composition of claim 1, wherein at least one of said detectable labels and at least one of said quenching moieties is located within the stem of said hairpin structures.

47. A method for detecting a target nucleic acid sequence, comprising:

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contacting a sample containing a mixture of nucleic acid molecules with at least one oligonucleotide, the oligonucleotide capable of hybridizing with a target nucleic acid molecule and comprises a detectable moiety, wherein the detectable moiety undergoes a change in one or more observable property upon hybridization to the target nucleic acid molecule; and

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observing the observable property, wherein a change in the observable property indicates the presence of the target nucleic acid sequence.

48. A method of determining the presence or absence of at least one particular nucleotide of interest at a specific position in a target nucleic acid molecule, comprising.

providing at least one target nucleic acid molecule having said nucleotide of interest at a specific position;

5 contacting said target nucleic acid molecule with at least one oligonucleotide, wherein at least a portion of the oligonucleotide is capable of forming base pairs or hybridizing with at least a portion of the nucleic acid molecule and wherein the oligonucleotide comprises at least one specificity enhancing group and/or at least one label; and

10 contacting the oligonucleotide and the target nucleic acid molecule with a polymerase less able to extend the oligonucleotide when the 3'-most nucleotide of the oligonucleotide does not base pair with the target nucleic acid and more able to extend the oligonucleotide when the 3'-most nucleotide of the oligonucleotide base pairs with the target nucleic acid molecule.

15 49. The method of claim 48, wherein the polymerase enzyme is *Tsp* DNA polymerase.

50. The method of claim 48, wherein the group is a fluorescent moiety.

20 51. The method according to claim 48, wherein the group is attached to a nucleotide at or near the 3'-nucleotide.

25 52. The method according to claim 48, wherein the group is attached to one of the ten 3'-most nucleotides.

53. The method according to claim 48, wherein the group is detectable.

30 54. The method according to claim 48, wherein the oligonucleotide is in the form of a hairpin.

55. A method for synthesizing or amplifying one or more nucleic acid molecules comprising:

5 mixing one or more nucleic acid templates or targets with one or more oligonucleotides, wherein said one or more of said oligonucleotides comprises at least one hairpin structure; and

incubating said mixture under conditions sufficient to synthesize or amplify one or more nucleic acid molecules complementary to all or a portion of said templates or targets.

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